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Review Article

RNA interference; A tool for treatment of human diseases

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Abstract

RNA interference is a powerful and rapid technique to knockout the gene expression by introducing either short interfering RNA or double stranded RNA fragments into targeted host. These RNA fragments degraded specific homologous mRNA. RNAi technology used as an essential tool in several model organisms such as invertebrates, mouse and fly to explore the role of individual gene. In recent era, RNAi is directly used in therapy of various infectious and non-infectious diseases. Variety of human diseases like cancer, viral and neuromuscular has been controlled through RNAi. Some vectors and inducible systems are available now to treat numerous disorders. This review paper provides the basis for further development of RNAi technology as a drug therapy against various human disorders.

Key words: RNA interference, cancer therapy, neuromuscular disorder therapy, antiviral therapy

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INTRODUCTION

Gene silencing occurs either through repression of transcription (DNA degradation) called transcriptional gene silencing (TGS) or translation repression (mRNA degradation), called posttranscriptional gene silencing. RNA interference (RNAi) is a gene silencing mechanism in case of animals, post transcriptional gene silencing (PTGS) in plants (Wassenegger and Pelissier, 1998) while quelling in fungi (Napoli *et al*., 1990; Fire *et al*., 1998; Matzke *et al*., 2001) that regulates gene expression and chromatin states and mediates genome defense mechanisms against transposons, transgenes and viruses. Hence, the suppression of a particular gene occurred by introducing dsRNA. Wingard published the first paper of RNA silencing in which he described that tobacco ring spot virus infected the tobacco plants and the originally infected leaves had necrotic symptom whereas the upper leaves showed immunity to the virus and resistant to secondary infection but information regarding to the mechanisms was not known in 1928 (Wingard, 1928). The RNAi phenomenon was first reported by Fire and his co-workers, when they injected *Caenorhabditis elegans* with the dsRNA (Fire *et al*., 1998) and got Nobel Prize in 2006. Tuschl *et al*. (1999) showed that RNAi also occurred in fruit flies, trypanosomes, plants, planaria, hydra and zebra fish.

RNAi used as a beneficial and therapeutic agent against numerous disorders such as infectious diseases, metabolic disorders and tumors (Angaji *et al*., 2010). RNAi appears to be a promising new therapeutic tool in the treatment for viral hepatitis, human immunodeficiency virus (HIV), cerebrovascular and cardiovascular diseases, metabolic disease, neuromascular and neurodegenerative disorders, and cancer (Angaji *et al*., 2010). RNAi also used to prevent inflammation and virus-induced tumorigenesis (Colbère-Garapin *et al*., 2005).

RNAi Mechanism

Mechanism of RNAi can be divided into three steps (Meister and Tuschl, 2004; Tomari and Zamore, 2005). The first step is the breakdown of long double-stranded RNA (dsDNA: 200-500 nucleotides) into short-interfering RNAs (siRNA: 21–26 nt) by a Ribonuclease III-like enzyme/ endoribonuclease or helicase called Dicer (Hamilton and Baulcombe, 1999; Bernstein *et al*., 2001). Highly conserved Dicers have been found in yeast,

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Drosophila, *C. elegans*, mice, plants and humans (Volpe *et al*., 2003; Bernstein *et al*. 2001; Knight *et al*., 2001; Bernstein *et al*., 2003; Golden *et al*., 2002; Zhang *et al*., 2002). They suggested that similar mechanism of RNAi pathways was shared by these organisms. These small fragments of 21 to 24 nucleotides are also called small interfering (siRNAs) and micro RNA (miRNAs). These molecules play role in gene expression regulation and cell growth control (Broderson and Voinnet, 2006; Malone and Hannon, 2009). In second step, these duplex siRNAs companion with RNA-induced silencing complex (RISC) protein of ~160 kDa. RISC comprises of Argonaute (Ago) proteins (Rand *et al*., 2005). Song *et al*. (2004) illustrated that eight members of Ago family were originate in humans, but only Ago2 possessed cleavage activity due to an active catalytic domain. Within the RISC these duplexes unwind and the one strand is degraded and removed by nucleases. Thirdly, the loaded ssRNA called guided strand, directs the RISC to the target mRNA (Hutvagner and Simard, 2008). Argonaute slices the phosphodiester bond and releases fragments of mRNA that are lastly degraded resulting in the gene silencing (Martinez and Tuschl, 2004; Schwarz *et al*., 2004) (Fig. 1).

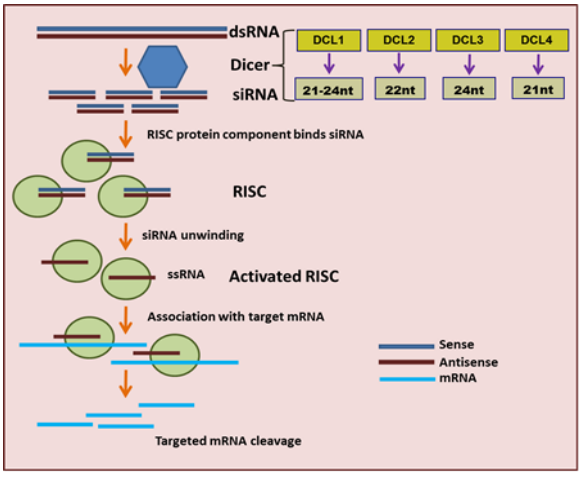

Figure 1: Mechanism of RNA silencing. (http://www.bioon.com/biology/Class422/1781.shtml)

Strategies for RNA interference

Two types of strategies for RNAi reagents delivery into mammalian cells can be used i.e. stable/inducible RNAi and transient RNAi (Paddison *et al*., 2002). Various delivery methods i.e. transfection, transduction, and bacterial transformation were used to deliver gene in host for RNAi. Different proteins (Dicers, RISC, AGOs) and promoters (RNA Polymerase III promoters i.e., H1, U6, and tRNA promoters) used to derive the RNAi. The viral based vectors like retroviruses (Barton and Medzhitov, 2002), adenoviruses and adeno-associated viral vectors (Li *et al*., 2006) have also been demonstrated for high-efficiency gene delivery.

Models used for RNAi

For functional genetic studies, model organisms have been importance. The nematode: *C. elegans,* fly: *D. melanogaster*, and vertebrate model: mouse are the most thoroughly used and implicit experimental animal for exploring human disease-related genes**.** Zebra-fish and puffer-fish also used for the RNAi (Buckingham *et al.,* 2004). Nematode are used as dominant genetic tool for learning neurobiology and development while drosophila is more powerful model for physiological studies rather than *C. elegans*. The genomes of these organisms have been fully sequenced and available for physiological and genetic data analysis. Today number of cell line of drosophila are available for functional analysis (Towers and Sattelle, 2002). Other organisms like trypanosomes, plants, planaria, and hydra were also used for RNAi studies (Tuschl *et al*., 1999).

Role of RNAi in various disorders

Cancer is targeted for RNAi-based therapy. The use of RNAi to silence the specific gene involved in disease pathogenicity holds the promising technology for the development of new therapies. Because all mammalian cell types have RNAi pathway and the primary challenge for effective gene silencing is the siRNA delivery to the appropriate organ lead to degenerate targeted mRNA (Dykxhoorn and Lieberman, 2006). Several *in vivo* studies have shown that RNAi based drugs can be effective against Huntington's disease, hypercholesterolemia, hepatitis, and cancer (Giladi *et al*., 2003; Xia *et al*., 2004; Soutschek *et al*., 2004; Diaz-Hernandez *et al*., 2005; Santel *et al*., 2006; Judge *et al*., 2009). Several *in vitro* and *in vivo* studies demonstrated that RNAi based therapy could be potential to treat single gene disorders and those with overexpression of proteins (Dykxhoorn and Lieberman, 2006). However, the delivery of siRNA to the target tissues is major challenge in RNAi-based therapeutics development for cancer. Cancer is the major target for RNA gene silencing.

Oncogenes and other tumor contributing genes are potentially targeting genes for gene suppressing by RNAi. RNAi-mediated genes reduced the side effect chances and also supplementary with chemotherapy. Numerous benefits of RNAi based therapeutics involves the target of multiple genes and the development of adapted anti-cancerous drugs that appropriate for the patient. RNAi targeted essential kinesin spindle protein (KSP) and polo-like kinase 1 (PLK1) to develop as a cancer therapy. They are cell cycle proteins i.e. involved in mitosis and cytokinesis (Judge *et al*., 2009). Similarly, pleiotrophin and protein kinase N3 has been validated for cancer therapy (Grzelinski *et al*., 2006; Leenders *et al*., 2004). Various sponsors such as Calando Pharmaceuticals, Silence Therapeuics AG, Alnylam Pharmaceuticals, Silences Ltd, Satris Phrama and Enzon Pharmaceuticals, Gradalis Inc., Duke University, Polish academy of sciences reported the clinical studies of targeted genes for cancer therapy.

The targeted genes were vascular endothelial growth factor (VEGF), M2 subunit of ribonucleotide reductase (RRM2), protein kinase N3 (PKN3), V-ki-ras2 Kirsten rat sarcoma viral oncogene homolog (KRAS), kinesin spindle protein (KSP), HIF-1 (hypoxia-induced Factor), MECL1 (Multicatalytic Endopeptidase Complex-Like 1), PLK1 (polo-like kinase 1), LMP2 (*latent membrane protein 2)*, LMP7 9 *latent membrane protein 7)*, Tenascin-c, Bcr-Abl (breakpoint cluster region- Abelson), and Survivin (Bora *et al*., 2012; Mansoori *et al*., 2014). RNAi knockdown the Duchenne muscular dystrophy (DMD) causing dystrophin gene by using homologue of *C. eleagans* (dys-1) and human dystrophin gene (dmDp186) in Drosophila (Grisoni *et al*., 2002). RNAi inhibited hepatitis B virus in animal model successfully (McCaffrey *et al*., 2003). Similarly, Giladi *et al*. (2003) also demonstrated the inhibition of hepatitis B viral replication through RNAi. Mohmmed *et al*. (2003) illustrated the use of RNAi to control malaria using a mouse malaria model (*Plasmodium berghei*). Numerous toxic viruses such as hucoronavirus, man immunodeficiency virus (HIV), hepatitis C and B viruses (HCV & HBV), human papillomavirus (HPV), and influenza A virus (IAV) were eliminated by RNAi (Ma *et al*., 2007).

CONCLUSION

It can be concluded that RNAi is genome based therapeutic technology. Hopefully this type of therapy will work as most efficient method for various human disorders.

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